Experimental Studies on the Efficacy of T1sr and T1/44 Vaccine Strains of *Mycoplasma mycoides* Subspecies *mycoides* (Small Colony) against a Field Isolate Causing Contagious Bovine Pleuropneumonia in Kenya - Effect of a Revaccination

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Key words

Cattle - Contagious bovine pleuropneumonia - Vaccine - Kenya

Summary

Contagious bovine pleuropneumonia (CBPP) is an important disease of cattle causing serious losses to the livestock industry in Africa. It is controlled primarily through vaccination. However, recent observations in the field indicate that the vaccine in current use failed to protect cattle in the face of outbreaks causing concern to the veterinary authorities of affected countries. A field trial was carried out to determine the causes for vaccine failure. T1/44 and T1sr vaccine strains of *Mycoplasma mycoides* subsp. *mycoides* (small colony) were used to vaccinate a group of 40 cattle per strain. Half of these cattle were challenged at three months and the other half at 15 months following vaccination, respectively. Half of the cattle used in the second trial were revaccinated one year after the primary vaccination. A dose of 10^7 mycoplasmas per milliliter was used in primary as well as secondary vaccinations. In the first challenge (at three months post the primary vaccination), the efficacy was 68.2 and 59% for T1sr and T1/44 vaccines, respectively. In the second challenge, the efficacy was 80.5 and 95.5% for T1sr and T1/44 vaccines, respectively, in cattle vaccinated twice, while in cattle vaccinated once and challenged at 15 months post primary vaccination the efficacy was 28.7 and 78.2% for T1sr and T1/44, respectively. These results confirm what had been obtained in previous trials but they also bring valuable new information. They show that a single vaccination, whatever the strain used, at the minimum required dose does not give satisfactory protection and that full protection can only be achieved by revaccination. Consequences for future vaccine trials and research needs are discussed.

■ INTRODUCTION

Contagious bovine pleuropneumonia (CBPP) is an important disease of cattle in Africa caused by *Mycoplasma mycoides* subsp. *mycoides* biotype small colony (MmmSC) and characterized by extensive lesions of pleurisy and pneumonia (13). Among infectious diseases that threaten African cattle, its importance is only second to rinderpest (RP) and, as more and more countries declare being free of RP, CBPP is likely to become the major priority for Veterinary Services in that continent.

In Africa, the control measures for CBPP are mainly based on vaccination with an empirically attenuated strain (15). Eradication has recently been achieved by stamping out policies in Botswana (11) but its cost has been tremendous and it is unlikely that other countries in Africa can afford it. Cattle owners often use antibiotic treatments in the field but officially these treatments are discouraged or forbidden because of the fear that they can promote the emergence of chronic carriers, resistant mycoplasma strains and antibiotic residues in human food. Therefore, control policies by vaccination seemed to be the obvious choice. It had been applied extensively in the past in many countries between the 60s and the 80s (2, 8), leading to the control or the eradication of the disease in many African countries after several years of repeated vaccination campaigns.

However, in the 90s CBPP has reemerged as a major disease. It reappeared in countries where it had been eradicated such as Rwanda, Kenya, Tanzania, Mauritania, and the number of outbreaks increased in countries where it was previously under control (10). This reemergence could well be the consequence of

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many factors such as the stop of the rinderpest vaccination campaigns that permitted the concomitant vaccination against CBPP in West Africa or the lack of preparedness in countries that had not been confronted to the disease for many years. More alarmingly some experts suggested that this reemergence could well be the consequence of a lack of protection afforded by the vaccinal strain T1sr as compared to the strains used in the past, former stocks of T1sr or T1/44.

This is why it was decided to conduct a comprehensive vaccination trial under the framework of EU/DG[1] development funding and the supervision of OAU/IBAR[2], Nairobi. These trials had a precise objective: evaluate the protection afforded by a single injection at the minimum dose required by international standards, i.e., 10^7 viable mycoplasmas per dose (14) for T1sr and T1/44 strains by a contact challenge performed three months after the vaccination. These trials were to be performed in three different African locations, Cameroon, Kenya and Namibia, in order to take into account the genetic variability of the MmmSC strains that could be correlated to a modification of the potency of the vaccine (1, 17). In addition, a second protocol was designed in Kenya to assess the protection afforded one year after vaccination. This protocol was subsequently modified after initial results showed a very low protection with a challenge three months after the vaccination. The modification included the revaccination of some animals in order to assess the subsequent protection afforded.

■ MATERIALS AND METHODS

Schematic Trial Protocol

- Purchase of susceptible animals;

- Initial pathogenicity testing of three MmmSC strains;

- Vaccination of 40 cattle with T1sr vaccine and 40 with T1/44 at 10^7 per dose.

Three months later:

- Intubation of 40 animals to serve as donors. They were put into contact with 40 unvaccinated control animals. Twenty T1sr vaccinated and 20 T1/44 vaccinated animals;

- Follow-up of these animals for two months after the initial clinical signs in the control group;

- Termination of the experiment by necropsy of all animals.

Twelve months later: Revaccination of 10 animals with T1sr and 10 animals with T1/44 (at the minimum 10^7 dose).

Fifteen months later (three months after revaccination): Intubation of 40 animals to serve as donors. They were put into contact with 40 unvaccinated animals. Ten animals vaccinated once with T1sr, 10 animals vaccinated once with T1/44, 10 animals vaccinated twice with T1sr and 10 animals vaccinated twice with T1/44.

Animals

Cattle were purchased from Kakamega District of Western Province, Kenya, historically known to be free of CBPP. Before purchase, 100 cattle from various homes in the district were randomly sampled for serum and found free of antibodies against MmmSC by the complement fixation test (CFT), the OIE[3] official serological test (14), and by cELISA (7), the new alternative test accepted by OIE. Following this, 255 cattle from the district were sampled and purchased for use in the experiment. The animals were transported to the experimental site at KARI[4] Muguga at the beginning of the rainy season. On arrival at the experimental site, the cattle were vaccinated against foot and mouth disease, lumpy skin disease, black quarter, anthrax and Rift Valley fever.

Initial Pathogenicity Trial

Fifteen animals were randomly divided into three groups of five animals. Each group was kept in a different house separated by a distance of 40 m. Each group was inoculated by a different strain, group one with the Gladysdale strain which had been traditionally used in former trials, group two with an MmmSC strain isolated in Rwanda in 1994, and group three with a local Kenyan isolate. The Gladysdale strain, originally isolated from Australia, had been kept at NVRC[5]-KARI since 1960 and the field isolate from Rwanda was isolated and characterized by CIRAD-EMVT[6] for the official confirmation of CBPP in that country. For the first two groups, the inoculation was performed intratracheally with 60 ml of an MmmSC pure culture followed by 30 ml of 1% suspension of low melting agar. Group three was inoculated intratracheally with 30 ml of a lung suspension obtained from a natural case of CBPP followed by the inoculation of 100 ml of normal saline. All animals were observed for eight weeks after which period the survivors were slaughtered.

Vaccination

Forty animals were vaccinated subcutaneously in the middle of the neck with T1sr at the dose of 10^7 viable mycoplasmas per animal; 40 animals were similarly vaccinated with T1/44. The vaccine batches were supplied by CIRAD-EMVT after they had successfully passed quality control at PANVAC[7], Debre Zeit, Ethiopia. After vaccination, the local reaction was thoroughly checked every day and the diameter of the swelling site evaluated by palpation. One year later, after the initial challenge had been completed, ten animals were randomly selected from each vaccinated group and subsequently revaccinated with the same vaccine batches, T1sr or T1/44, under the same conditions.

Intubation Protocol and Challenge by Contact

Each animal was sedated with 2 ml of Chanazine 2% (2% xylocaine) injected intramuscularly. Fifteen minutes later the animal was made to lie on its right side and a one meter long, 10 mm wide in diameter, tube was inserted into the trachea. A narrower tube, 1.5 m long and 2 mm wide, was inserted into the wider tube and pushed into the trachea. The narrower tube was used to inject the infective preparation. In the first trial performed three months after the initial vaccination, the inoculum consisted of 60 ml of a pure culture of the Kenyan isolate followed by 30 ml of a lung suspension in normal saline. In the second challenge performed 15 months after the initial vaccination, the lung suspension was replaced by 30 ml of 1% agar suspension.

The group of intubated animals was observed for one week and the contact was performed when the first signs of disease appeared in that group, as measured by hyperthermia. Forty naive animals, 20 animals vaccinated with T1sr and 20 animals vaccinated by T1/44, were introduced into the premises occupied by the intubated animals and kept in close contact thereafter.

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2. Organization of African Unity/Inter-African Bureau for Animal Resources
3. Office international des épizooties
4. Kenya Agricultural Research Institute
5. National Veterinary Research Centre
6. Centre de coopération internationale en recherche agronomique pour le développement, département d'élevage et de médecine vétérinaire
7. Pan African Vaccine Laboratory

Follow-up of Animals

Rectal temperatures were taken daily. Serum samples were harvested before the challenge and every other week after vaccination. In the second experiment, animals were bled monthly after the vaccination, then every other week after the beginning of the challenge. During each challenge, the animals that survived the disease were slaughtered two months after the initial clinical signs had been observed in the control group, namely fever for three consecutive days. Clinical signs and lesions were noted and transcribed into a pathological score as described by Hudson and Turner (5) in order to calculate a protection rate. Briefly, this pathological score is calculated as follows:

- The presence of only encapsulated resolving or fibrous lesions or the presence of pleural adhesions only are rated 1;

- If other types of lesions are present, namely consolidated, acute, necrotic or sequestrated, these lesions are rated 2;

- This initial score is then multiplied by a factor depending on the size of the lesions: 1 (the lesion size is under 5 cm), 2 (it is over 5 and under 20 cm) and 3 (it is over 20 cm).

Hence the maximum pathology score is 2 x 3 = 6.

Serum samples were analyzed by CFT, competition ELISA, but also by indirect ELISA as described previously (19).

■ RESULTS

Postvaccinal Reaction

Some swelling occurred at the injection site between days 6 and 29. None of them occurred in the T1sr group (swelling less than 2 cm in diameter). Conversely, there were 15 animals in the T1/44 group that exhibited a swelling of more than 2 cm in diameter. Five of them had noticeable swellings (more than 6 cm in diameter) and one developed a severe invading necrosis at the shoulder, together with fever and was subsequently slaughtered. A mycoplasma strain was isolated from the skin lesion and identified as a T1 vaccinal strain by a specific polymerase chain reaction (9).

Initial Pathogenicity Trial

All five cattle infected with the Kenyan inoculum (made of lung suspension) showed clinical signs of CBPP and lesions from which MmmSC was isolated again. Comparatively, the other inoculum failed to reproduce CBPP, therefore it was decided to use the Kenyan isolates for the subsequent challenges.

Intubation and Transmission Success

This success can be evaluated by analyzing the temperature, the serological results and the lesions found after death or slaughter.

Temperature

The temperature of healthy animals was always below 39°C as animals with CBPP had intermittent fever with temperatures ranging from 39.5 to 40.5°C. The number of animals exhibiting fever in each group at a defined day was used to monitor the efficacy of disease uptake after intubation or disease transmission to the control groups. In the first trial intubation was quite successful but the rate of transmission to the control group was rather low. In the second experiment the opposite situation prevailed, intubation transmitted CBPP in only five animals but these five animals contaminated the control group very efficiently. Based on the data on animals exhibiting fever in the different groups, the incubation period lasted 57 days in the first experiment and 40 days in the second.

Serology

During the first trial the five animals that exhibited fever in the intubated group also exhibited a seroconversion as measured by indirect ELISA except for one animal that died rapidly, 20 days after inoculation. Conversely four animals exhibited a seroconversion while not exhibiting any fever after intubation. None of these animals displayed any lesion nor any fever afterwards either, an indication that they were probably protected by the initial intubation. Out of the 31 animals left, 14 (45%) did not exhibit any fever afterwards as compared to 11 out of 40 (27%) in the control group. This difference seems to indicate that some of the intubated animals did not contract CBPP nor exhibit any seroconversion were nevertheless protected. Protection may not be strictly correlated with seroconversion as measured by this indirect ELISA.

In the control groups, seroconversion took place after the initial occurrence of fever but it must be noted that temperature was recorded daily and blood samples were taken only every 15 days. The precise date when an animal was seroconverting was impossible to obtain. However, in three cases seroconversion was seen prior to the observation of fever. As seroconversion necessarily follows multiplication of MmmSCs, these results showed that fever may not be triggered by multiplication of mycoplasmas but simply reflects the intensity of lesions. This hypothesis may be substantiated by the analysis of the lesions observed after necropsy.

Necropsy and Pathology Index

To investigate if there was any correlation between fever and intensity of lesions, it was decided to cumulate for each animal the number of days with fever during the whole trial and to compare these data with the lesions observed in the end, as measured by the Hudson and Turner index. Animals were grouped according to their pathology index and, in each group, the distribution of the number of days with fever analyzed. It is clear that there is an overall correlation between the intensity of lesions and the number of days with fever (Table I). The mean day numbers with fever in each group is strictly correlated with the pathology index. However, this correlation is not always true at the individual level as some animals exhibiting a high pathology score (4 or 6) apparently did not exhibit many days with fever.

Evaluation of Protection

The protection rate was calculated according to Hudson and Turner by comparing pathological scores in the vaccinated and in the control groups. A single injection gave apparently a similar protection, regardless of the type of vaccine, 59% for T1/44 and 68.2% for T1sr (Table II). Similarly, the protection afforded by two vaccinations at a one-year interval was very good, regardless of the type of vaccine, 80.4% for T1sr and 95% for T1/44 (Table III). A marked difference was noted when the challenge was performed 15 months after a single vaccination. The protection afforded by the T1sr dose dropped at 28%, whereas the protection afforded by the T1/44 was maintained at a relatively high level, 78,2% (Table III).

It is noteworthy that the intensity of the challenge was quite mild during the first trial, with a mean pathology score of 2.2 in the control group, but very high in the second trial, with a mean pathology score of 4.6. This last result indicates that the protection rates observed in the second trial are very robust as it is unlikely that animals in the field will be confronted to such a harsh challenge.

Table I

Correlation between the pathology index and the number of days with fever in the control group of the second trial

Num. of days with fever	Hudson and Turner pathology score			
	6	4	2	0
< 0	1	4	3	22
0 to 5	1	11	2	3
5 to 10	5	8	1	0
10 to 20	11	6	0	0
> 20	1	0	0	0
Mean	12	6.1	2.5	0.12

Table II

Summary of the protection rates in cattle vaccinated once with the minimum recommended dose (10^7) of T1sr and T1/44 and challenged three months later by contact with intubated animals

	Number of cattle	Mean pathology score	Standard deviation	Protection rate
Control	40	22	23	
T1sr	20	7	15	682
T1/44	20	9	18	59

Table III

Summary of the protection rate in cattle vaccinated once with T1sr or T1/44 and challenged 15 months later and cattle vaccinated twice at one-year interval and challenged three months after the second injection

	Number of cattle	Pathology score	Standard deviation	Protection rate
Control	41	46	14	
T1sr once	11	33	24	28.7
T1/44 once	8	1	19	78.2
T1sr twice	10	9	14	80.4
T1/44 twice	10	2	6	95.5

■ DISCUSSION AND CONCLUSION

There was an obvious difference between the two trials in Kenya in terms of transmission success. In spite of an apparent success of intubation in the first trial, the transmission to the contact animals did not occur as rapidly as in the second trial. This was quite surprising as apparently the intubation was less successful in the second trial with only five animals being infected primarily. This difference cannot be explained by trivial factors as the same type of animals and the same challenge strain was used. The only explanation should be found in additional factors that differed between the two trials. The body condition could be one of them, this body condition was not very good in the first trial as the animals had been inadvertently infested by liverfluke during grazing before the experiment and fed with lower quality fodder during the experiment. If this were the case, it would mean that a good body condition would be a predisposing factor for CBPP. This could be explained by the fact that during a CBPP outbreak, as lesions are triggered by an overwhelming immune response, only the animals capable of developing such a response are prone to develop lesions. An additional factor can be found in the immunomodulation effect of some parasitic infestation such as liverfluke. This factor has already been mentioned for trypanosomes (6). All these factors may certainly also play a role in the ability of the vaccine to induce a good protection, something which is often overlooked in the field and may explain some apparent vaccination failures.

The classical Hudson and Turner score was used here to measure the protection rate as it has frequently been the case in the past (3), ensuring valid comparisons with former trials. It must be noted that the present trial was slightly different from former ones as all animals were slaughtered rapidly at the end of the experiment due to time constraints. In former trials, animals were slaughtered after a defined period of time following seroconversion. This slight difference is unlikely to modify dramatically the final results because of the rapid transmission of the disease. The Hudson and Turner scoring system may not be completely satisfactory, the authors themselves had mentioned that “the procedure for calculating scores is arbitrary, and may well be susceptible to some improvement” (5). The “total score” includes parameters such as fever and pathology scores that are correlated, an indication that a simplification of the scoring system could be achieved. More importantly, means are calculated on distributions of results that are obviously not Gaussian leading to statistical irrelevancies. In the future it may be advisable to use comparisons with other tests such as Chi^2 and to give more weight to animals that die or suffer from important lesions.

The constant problem encountered with CBPP experimental trials is finance. Due to the absence of a validated *in vitro* or laboratory animal model, all experiments have to be performed on cattle. The use of mice as laboratory models (16) has to be questioned as virulence factors of MmmSC and protection in cattle certainly depend on specificities of the bovine immune response. The relative difficulty to reproduce the disease by intubation necessitates the use of a large group of intubated animals. In addition, the somewhat unpredictable rate of transmission success also calls for large control groups and treated animals so that statistically meaningful results may be obtained. This is the reason why experimental trials have to focus on specific objectives. The relative high cost of these trials have to be put in perspective with the probable high cost of the disease itself, but also with the unacceptable high cost of vaccination programs if they are performed with inadequate vaccines.

The protection afforded by a single vaccination in Kenya was quite low 60%. A similar experiment in Cameroon reached a similar conclusion with an even lower protection rate, 30% (18, 19). The difference may be explained by the harsher trial in Cameroon but the conclusion is nevertheless the same. It was expected that the maximum protection rate would be obtained three months after vaccination, therefore protection was not satisfactory.

Retrospectively, it explains why an emergency vaccination alone was not able to stop the progress of CBPP when it invaded Botswana. The present results show clearly that the choice of vaccinal strains was not the reason for failure as both vaccinal

strains, T1sr and T1/44, gave similar protection rates at the minimum required dose.

The second experiment at KARI established interesting additional data. First, it showed that there was a significant difference between T1/44 and T1sr in terms of duration of protection, T1/44 still induced a protection 15 months after vaccination whereas T1sr did not. In addition, the T1/44 protection rate apparently increased. This may be an indication that the time needed for the establishment of protection might be longer than three months. However this good result must be balanced by the fact that T1/44 also induced a significant number of postvaccinal reactions, one of them leading to the death of the animal. Second, it also showed that a revaccination dramatically increased the protection rate to a satisfactory level, 80 to 95%, regardless of the vaccinal strain. It must also be noted that revaccination with T1/44 did not induce any postvaccinal reaction.

These results fully reestablish the potency of the two vaccinal strains T1sr and T1/44 at the minimum required dose when animals are confronted to local pathogenic strains, the original objective of the studies. A single vaccination induced a limited protection and satisfactory protection could be achieved only by revaccination. These results are quite similar to what was known 30 years ago (13), and they stress that only repeated vaccination campaigns can achieve an acceptable protection rate in cattle populations. They clearly specify what can be expected from the minimum required dosage as very few, if not just one, previous works had investigated this question (4). Future research should focus on specific important points that will rapidly allow a better definition of vaccination strategies. Two of them can already be identified: first, establish if there is a dose response for an initial vaccination and, second, specify the conditions for revaccination timing in order to obtain the longest lasting protection. The dose response trial is important as its conclusion may modify the existing recommendations for minimal dosages in the vaccines. If there is really a dose response, emergency vaccinations could be performed with increased dosages in order to achieve a more rapid and efficient protection. In addition, the current standards apply to vaccines at the production site and do not take into account the very probable loss of titer that will frequently occur in the field, a reason why some authors advocate higher minimum standards (12). In the present situation revaccinations seem to be a prerequisite for the establishment of a satisfactory immunity. However, the timing of these revaccinations and the duration of immunity have not been clearly established yet.

Owing to budget constraints that are always increasing, it is quite unlikely that many African countries will be able to perform massive repeated vaccination campaigns. It is therefore clear that a cost-benefit analysis of the different control strategies will have to be done, taking into account the real prevalence and incidence of CBPP in each case. This naturally calls for a preliminary evaluation of the prevalence of CBPP with the help of epidemiological and statistical tools that will permit minimizing the cost. To avoid expensive trials and errors in the field, modelization of CBPP transmission may be the cheapest means to validate each strategy. In any case CBPP vaccines that would be both innocuous and more effective are utterly needed.

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Résumé

Wesonga H.O., Thiaucourt F. Etudes expérimentales sur l'efficacité des souches vaccinales T1sr et T1/44 de *Mycoplasma mycoides* spp. *mycoides* (petite colonie) contre un isolat causant la péripneumonie contagieuse bovine au Kenya - Effet d'une infection de rappel

La péripneumonie contagieuse bovine est une maladie provoquant de lourdes pertes dans les élevages bovins d'Afrique. Son contrôle passe habituellement par la vaccination. Des observations récentes ont cependant montré que les campagnes de vaccination sur le terrain actuelles ne sont pas suffisantes lors de l'apparition de nouveaux foyers, situation préoccupante pour les responsables vétérinaires des pays concernés. Un essai vaccinal a été réalisé sur le terrain afin de déterminer les causes de ces échecs vaccinaux. Les souches vaccinales T1sr et T1/44 de *Mycoplasma mycoides* spp. *mycoides* (petite colonie) ont été utilisées pour vacciner des groupes de 40 bovins par souche. L'épreuve virulente a été réalisée respectivement chez la moitié des animaux trois mois après la vaccination et chez l'autre moitié 15 mois après celle-ci. La moitié des animaux utilisés lors de la deuxième épreuve ont reçu un rappel un an après la primovaccination. Les vaccinations ainsi que les rappels ont été effectués à des doses de 10^7 mycoplasmes par millilitre. Au cours de la première épreuve virulente (à trois mois après la vaccination initiale), les protections mesurées ont été de 68,2 p. 100 pour la souche T1sr et de 59 p. 100 pour la souche T1/44. Lors de la deuxième épreuve, les protections mesurées chez les animaux ayant reçu une dose de rappel ont été de 80,5 p. 100 pour T1sr et de 95,5 p. 100 pour T1/44. Pour ceux n'ayant reçu qu'une seule dose vaccinale 15 mois auparavant, la protection mesurée a été de 28,7 p. 100 pour T1sr et de 78,2 p. 100 pour T1/44. Ces résultats confirment ceux qui avaient été observés lors d'essais antérieurs mais ils apportent également des éléments d'information nouveaux et importants. Il est en particulier confirmé qu'une primovaccination avec une dose minimale ne confère pas de protection satisfaisante, quelle que soit la souche vaccinale utilisée. En revanche, une protection totale ne peut être atteinte qu'après un rappel. Les conséquences pour des essais de vaccination ultérieurs et les orientations à prendre dans la recherche sont discutées.

Mots-clés : Bovin - Péripneumonie contagieuse bovine - Vaccin - Kenya.

Resumen

Wesonga H.O., Thiaucourt F. Estudios experimentales sobre la eficiencia de las cepas de vacunas T1sr y T1/44 de *Mycoplasma mycoides* subespecie *mycoides* (colonia pequeña) contra un aislamiento de campo causante de la pleuroneumonía contagiosa bovina en Kenia. Efecto de la revacunación

La pleuroneumonía contagiosa bovina (CBPP) es una enfermedad importante en el ganado, causante de serias pérdidas en la industria pecuaria en Africa. Se controla principalmente a través de la vacunación. Sin embargo, recientes observaciones de campo indican que la vacuna actualmente en uso no logra proteger al ganado en caso de brotes, preocupando a las autoridades veterinarias de los países afectados. Un estudio de campo fue llevado a cabo, con el fin de determinar las causas del fracaso de la vacuna. Las cepas de vacunas de T1/44 y T1sr de *Mycoplasma mycoides* subespecie *mycoides* (colonia pequeña) se usaron para vacunar un grupo de 40 animales por cepa. La mitad de este ganado se infectó a los tres meses y la otra mitad a los 15 meses post vacunación, respectivamente. La mitad del ganado utilizado en el segundo experimento fue vacunado nuevamente un año después de la primera vacunación. Una dosis de 10^7 micoplasmas por mililitro se usó en las vacunaciones tanto en la primera, como en la secunda. En el primer estudio (tres meses después de la primera vacunación), la eficiencia fue de 68,2 y 59% para las vacunas de T1sr y T1/44 respectivamente. En el segundo estudio, la eficiencia fue de 80,5 y de 95,5% para las vacunas de T1sr y T1/44 respectivamente, para el ganado vacunado dos veces, mientras que en el ganado vacunado una sola vez y infectado a los quince meses después de la primera vacunación, la eficiencia fue de 28,7 y 78,2% para las vacunas de T1sr y T1/44 respectivamente. Estos resultados concuerdan con aquellos obtenidos en estudios anteriores y además aportan nueva y valiosa información. Muestran que una vacunación única, cualquiera que sea la cepa, a una dosis mínima, no provee protección satisfactoria y que una protección total puede obtenerse mediante una revacunación. Se discuten las implicaciones para los estudios e investigaciones de vacunación futuros.

Palabras clave: Ganado bovino - Pleuropneumonía contagiosa bovina - Vacuna - Kenia.